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Perspective

Implantable Electrochemical Sensors for Brain Research

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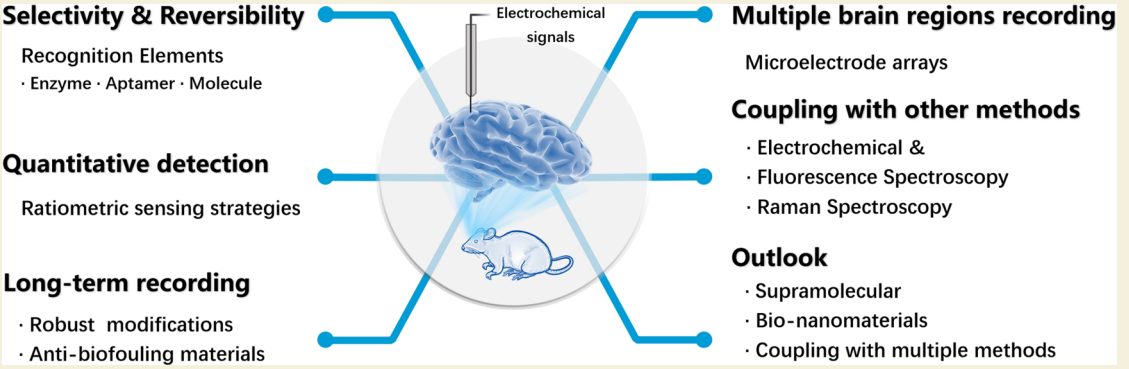

ABSTRACT: Implantable electrochemical sensors provide reliable tools for in vivo brain research. Recent advances in electrode surface design and high-precision fabrication of devices led to significant developments in selectivity, reversibility, quantitative detection, stability, and compatibility of other methods, which enabled electrochemical sensors to provide molecular-scale research tools for dissecting the mechanisms of the brain. In this Perspective, we summarize the contribution of these advances to brain research and provide an outlook on the development of the next generation of electrochemical sensors for the brain.

KEYWORDS: *in vivo, brain, biosensors, selectivity, long-term stability, electrical and chemical signals*

1. INTRODUCTION

Chemical signals such as ions, neurotransmitters, and reactive oxygen species (ROS) in the living brain are closely related to the physiological and pathological processes in living systems. Therefore, the development of in vivo brain sensors that can accurately and stably quantify chemical signals help humans understand the working mechanism of the brain.[1−4] Although optical methods such as fluorescent contrast agents for visible and infrared light have been developed extensively for brain imaging,[5,6] they are still limited by the depth of tissue penetration and tissue autofluorescence. In recent years, advances in materials science and instrumentation facilitated the development of implantable electrochemical microelectrode technology, which allowed scientists to monitor chemical signals in the brain with high spatial and temporal resolution.[7−10] Unfortunately, the current in vivo electrochemical sensors still face several challenges due to the complexity of the brain environment. First, it is hard to identify various electrochemical signals selectively through electrochemical redox reactions. Although identification elements such as enzymes and aptamers were developed to improve the selectivity of in vivo detection, the number of natural enzyme and aptamer species was still limited, which inhibited further exploration of a wider range of chemical signals.[11] Additionally, the identification process of these methods were usually irreversible, which made it difficult to continuously monitor the dynamics of the substance.[12,13] Second, the interfacial properties between the molecule and the electrode affected the stability of the electrode. Meanwhile, contaminants such as proteins in the brain tend to adsorb to the electrode surface, resulting in signal loss.[14] Finally, the additional voltage applied during electrochemical recording not only affects the neuroelectrical signal but also interferes with the electrophysiological recording.

This Perspective focuses on the development of design principles and strategies for high-performance implantable electrochemical sensors to address the above challenges. First, recognition elements such as enzymes and aptamers were designed and developed to improve selectivity in the complicated brain environments.[15−17] A double-recognition strategy[18] by designing organic molecules followed by electrochemical reactions was proposed, and a series of recognition organic molecules were designed and synthesized

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to monitor ROS, ions, etc., with high selectivity. Furthermore, a ratiometric sensing strategy was proposed to provide a built-in correction for the quantitative monitoring of substance concentrations in vivo with high accuracy. The systematic study of the ligand recognition mechanism made it possible to develop both highly selective and highly reversible Ca^{2+} sensors. Second, to stably immobilize the functionalized molecules on the electrode surface, a series of surface modification methods such as Au−S, Au−Se, Au−C≡C bonding, and bidentate thiols were systematically investigated. It was found that Au−C≡C bonding and bidentate thiols had both excellent electrochemical properties and stability in comparison. On the other hand, various materials such as hydrophilic and mesoporous materials have been reported to be resistant to biological contamination.[19−21] However, it is difficult to further modify functionalized recognition molecules on surfaces completely covered by biocontamination resistant materials. Recently, hydrophilic graphene oxide microbands were alternatively wrapped around the surface of gold particle-modified carbon fiber microelectrodes, and the exposed gold particles provided active sites for modification of recognition molecules.[14,22] Finally, to avoid the effects of applied voltages on neuroelectrophysiological and electrochemical recordings, potentiometric methods based on open-circuit potentials were developed for simultaneous recording of chemical signals and local field potentials (LFPs) of electrophysiology without crosstalk.[23] We also look forward to the next generation of implantable in vivo electrochemical tools, such as the designing of supramolecular recognition to further improve response speed and reversibility, the developing of new materials to reduce biological loss, and the coupling of multiple in vivo analytical methods with electrochemical sensors to provide more comprehensive biological information in the whole brain.

2. DESIGNING IDENTIFICATION ELEMENTS TO IMPROVE SENSOR SELECTIVITY AND REVERSIBILITY

Unlike the detection environment of in vitro artificial solutions, the living brain is rich in bioactive substances such as ions, neurotransmitters, amino acids, reactive oxygen species, and proteins. Therefore, improving the selectivity of in vivo sensors is the primary priority for in vivo brain detection. As early as 1973, Adams et al. implanted carbon paste electrodes directly into the rat brain to identify biogenic amines.[24] In 1988, Wightman et al. established fast scanning cyclic voltammetry (FSCV) for selective identification of dopamine.[25] Phillips et al. further extended this technique to real-time monitoring of dopamine release during human surgery.[26] Another attempt to improve selectivity was the development of catalytically active nanomaterials to reduce the overpotential of redox. For example, carbon nanotube fibers could accelerate the electron transfer process of ascorbic acid, resulting in selective determination of AA. Although many modifications of electrode surface properties to improve selectivity were reported, the accurate identification of a wider range of bioactive molecules still required the design of flexible and abundant identification elements. Although there were some reports indicating that selectivity could be improved by adjusting the surface properties of electrodes, developing recognition elements with flexibly designing could accurate detect more bioactive molecules in vivo.

2.1. Enzyme-Modified Electrodes

Natural enzymes can catalyze molecules with high selectivity. Taking advantage of this property, three generations of enzyme-based electrochemical sensors have been developed. Clark et al. developed the first generation of enzyme electrochemical sensors using O_2 as an electron acceptor.[27] The analytes were detected indirectly by quantifying the H_2O_2 obtained by enzyme catalysis. However, due to the high oxidation potential of H_2O_2, in vivo electroactive molecules such as ascorbic acid, uric acid, and electroactive neurotransmitters could interfere with the detection. To improve the selectivity, Baker et al. modified the surface of the microelectrode with an electropolymeric membrane to suppress the spread and oxidation of interfering molecules to the surface of electrode.[28] However, the dynamic changes of O_2 and H_2O_2 reduced the stability of the first generation enzyme sensors. In addition, the indirect determination of H_2O_2 limited the highly selective analysis of other reactive oxygen molecules.

Subsequently, second-generation enzyme sensors were developed using electron transfer mediators as electron acceptors for enzyme reactions. Compared to the high oxidation potential of H_2O_2, electron transfer mediators such as potassium ferricyanide, ferrocene, and quinone-type compounds had low redox potentials, thus reducing interference from other electroactive molecules.[29−32] Wang et al. developed a glucose sensor by dispersing enzyme oxide, ferrocene, and graphene oxide on a thin film to take advantage of the homogeneous film-forming properties.[33] The method combined with microdialysis technology enabled successful monitoring of glucose changes in the murine brain. However, the high toxicity of these electron transfer mediators and the low efficiency of electron transfer with the electrode surface made it difficult to be widely used in in vivo monitoring.

Taniguchi and co-workers first developed the third-generation enzyme sensor by modifying cytochrome *c* (Cyt *c*) on the surface of a gold electrode.[34] This design abandoned the electron transfer medium and immobilized the enzyme directly on the electrode surface to achieve direct electron transfer between the enzyme and the electrode. However, the method of Cyt *c* modification on the electrode surface affected the performance of the sensor. Tian's group designed a improved method to develop different gold nanostructures on the electrode surface.[35−40] Also, they systematically investigated the performance of Cyt *c* on pyramidal, rod, and spherical gold nanostructures.[35] They found that pyramidal and rod shapes can promote electron transfer of Cyt *c*, but spherical gold does not. In addition, Tian's group also developed a hydrogel-based method for enzymatic immobilization on the electrode surface, which improved the stability of Cyt *c* modification and enabled accurate detection of H_2O_2 release from living cells.[41]

Another important biological enzyme is superoxide dismutase (SOD), which can rapidly catalyze the disproportionation reaction of $O_2^{\bullet-}$ to generate O_2 and H_2O_2. Tian and Ohsaka first immobilized SOD on the electrode surface to develop a third-generation enzyme sensor for $O_2^{\bullet-}$ detection (Figure 1D).[15] They then designed self-assembled 3-mercaptopropionic acid (MPA) monolayers, gold nanoparticles and TiO_2 nanopins on the surface of gold electrodes to promote the electron transfer efficiency of SOD.[42−45] To avoid the effects of complex biological environments, Tian's group modified ZnO nanodiscs on the electrode surface. The SOD adsorbed on the ZnO nanodisk film can sense $O_2^{\bullet-}$ at 0 mV

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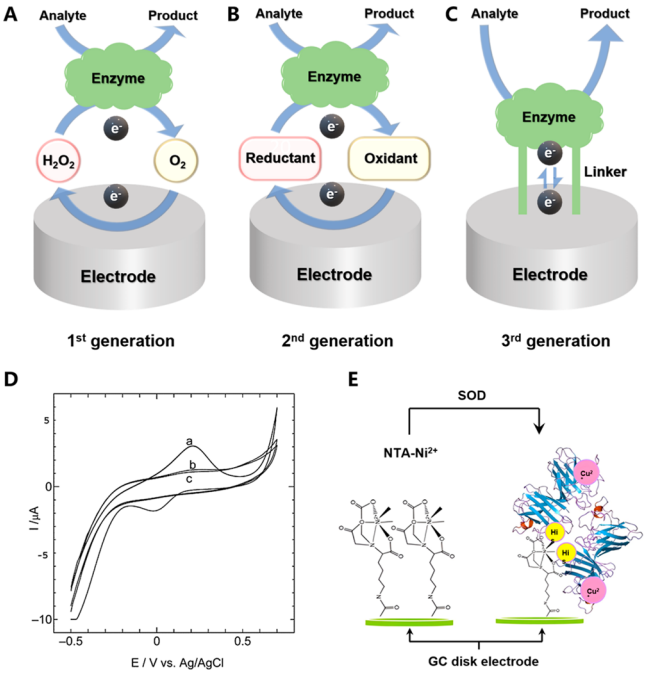

Figure 1. (A–C) Schematic representation of the principles of the first (A), the second (B), and third (C) generation enzyme-modified electrodes. (D) Cyclic voltammetry curves obtained at (a) SOD/Cys/Au, (b) bare Au, and (c) Cys/Au electrodes in PBS containing 0.002 units of XOD and 50 μM xanthine. Reproduced with permission from ref 42. (E) Schematic diagram of the modification of NTA and SOD on the electrode surface. Reproduced with permission from ref 47. Copyright 2013 Elsevier.

(vs Ag/AgCl), thus effectively avoiding the interference of H_2O_2, UA, AA, etc.[46] Although a range of nanostructures were developed to improve sensor performance, the in vivo brain detection environment strictly limited the size of the electrodes. Moreover, there were also challenges in modifying SOD stably on the electrode surface. To address these challenges, Tian's group immobilized SOD on the carbon fiber microelectrode using a hypoazotriacetic acid (NTA)/histidine labeling (HT) strategy (Figure 1E).[47] The sensor successfully monitored $O_2^{\bullet-}$ during ischemia–reperfusion in the rat brain.

Although the enzyme-modified electrode had a very high analytical selectivity, its recognition range was limited due to the scarcity of natural enzyme species. The synthesis of biomimetic enzyme structures using nanomaterials was one of the efficient ways to further expand the application of enzyme-modified electrodes. Luo et al. developed a Mn^{2+}/Nafion biomimetic SOD structure based on TiO_2 nanopins, which enabled live cells to be directly cultivated on the electrode surface, thus further improving the efficiency of electron transfer.[48] Such research paradigms were expected to be further applied to in vivo brain research in the future with design optimization.

2.2. Selection of Aptamers for Highly Selective Recognition

Deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) are the genetic basis of organisms. However, it was discovered that some short, single-stranded DNA and RNA sequences possessed the ability to bind specifically to certain substances. In 1990, an automated technique known as SELEX was introduced for the in vitro selective isolation of DNA or RNA fragments with high specific recognition properties for specific ions, molecules, proteins, etc.[49,50] The DNA sequence developed by SELEX was first used as an aptamer-based "turn-on" electrochemical sensor by Xiao's group. After that, Fan's group developed an electrochemical aptamer-modified sensor to detect ATP (Figure 2A).[51] In the presence of ATP,

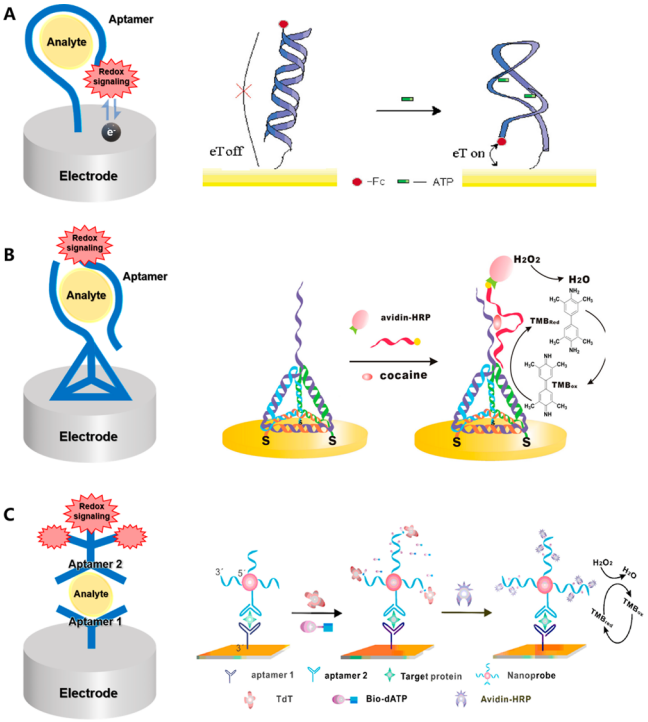

Figure 2. (A) Schematic diagram of ATP detection by aptamer-modified electrode. Reproduced with permission from ref 51. (B) Schematic diagram of gold electrode modified by DNA tetrahedron and aptamer. Reproduced with permission from ref 52. (C) Schematic diagram of the "sandwich" structure of aptamer-modified electrode and the principle of target molecule analysis. Reproduced with permission from ref 53. Copyright 2016 Elsevier.

the double chains melted and released their complementary chains, allowing the modified ferrocene to approach the electrode surface, thus producing an enhanced electrochemical signal. To address the induced perturbation resulting from local crowding of the aptamer modification on the electrode surface, Fan's group incorporated the high structural stability of DNA tetrahedra to construct aptamer tetrahedral DNA nanostructures on the electrode surface, resulting in a significantly lower detection limit of 33 nM (Figure 2B).[52] The development of combining aptamers and nanomaterials guided the direction of this technology. Zuo's group utilized a pair of DNA aptamers that simultaneously bound to carcinoembryonic antigen (Figure 2C), which was further amplified by modifying deoxynucleotide transferase (TdT) in aptamer 2.[53] Recently, Tang's group developed a 3D-printed photoelectrochemical aptamer-based sensor by combining antigens with upconversion nanoparticles,[54] while Zhang et al. comodified the aptamer with gold nanoparticles and Ti_3C_2MXenes, in which MXenes functioned as a reducing agent, substantially enhancing sensitivity in the analysis of exosomes.[55] In summary, aptamer-modified electrochemical sensors exhibited exceptional selectivity. However, the conformational changes occurring during recognition often

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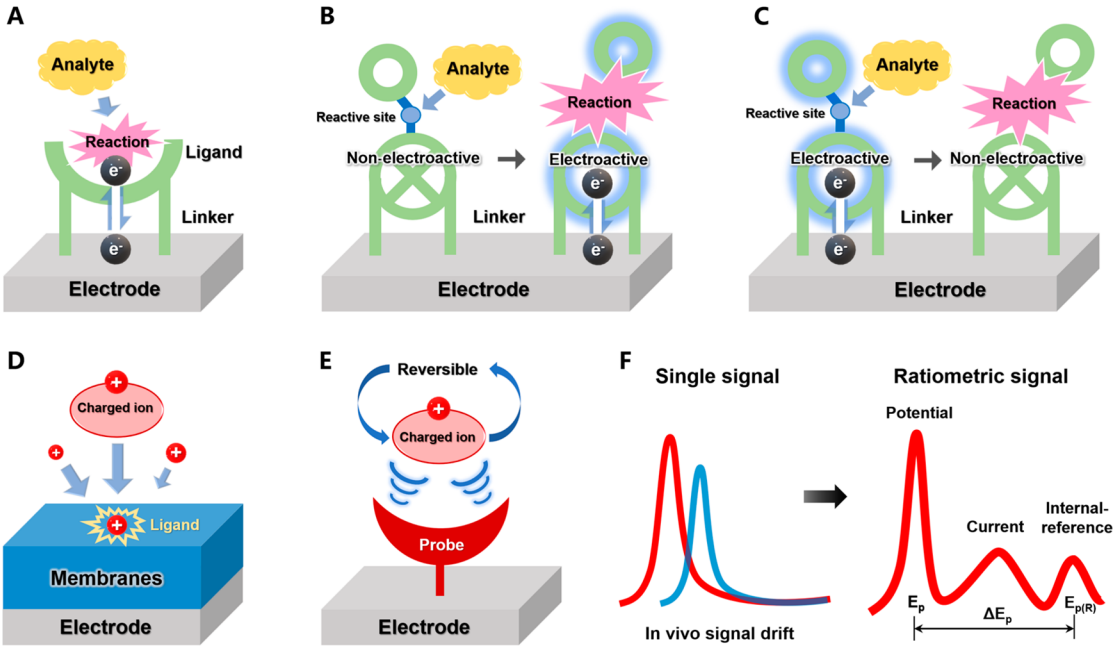

Figure 3. (A) Schematic diagram of double-recognition strategy, with chemical recognition followed by electrochemical reaction. (B, C) Schematic diagram of "turn-on" (B) and "turn-off" (C) electrochemical sensors. (D) Schematic diagram of ion-selective electrode based on PVC membrane. (E) Schematic diagram of sensor design with reversible recognition ligands on electrode surface. (F) Schematic diagram of ratiometric electrochemical sensor.

required a reaction time, leading to poor temporal resolution. In the living brain, changes in biologically active molecules, such as neurotransmitters, occurred in the millisecond range. Therefore, future development of aptamer sensors should incorporate more kinetic designs to compensate for this deficiency.

2.3. Double-Recognition Strategy for Selective Determination

Organic molecules emerged as promising recognition elements due to advancements in coordination chemistry and organic reactions. The flexible design of these molecules facilitated the development of a strategy that significantly improves the selectivity, stability, and responsiveness of sensors. Tian's team first proposed a double-recognition strategy, in which electrochemical reactions follow chemical recognition to enhance recognition selectivity (Figure 3A).[18] The electrode surface was modified with specific recognition components that could react specifically with the analyte to accurately identify the analyte from a multitude of interferents. Subsequently, the electrochemical reaction occurred under certain conditions such as a specific potential, thus enhancing the selectivity of in vivo analysis through double recognition. This universal method was not limited to "turn-on" sensors (Figure 3B), which produce enhanced electrochemical signals, but also "turn-off" sensors (Figure 3C), which produce reduced signals. One of the main principles of "turn-on" electrochemical sensors is the coordination recognition reaction of the analyte. Chai et al. designed and synthesized the organic ligand AE-TPAA, which selectively recognized Cu^{2+} in conjunction with the reduction peak signal of Cu^{2+} at −0.12 V (vs Ag/AgCl), allowing for the analysis of brain microdialysates during murine brain ischemia.[18] To investigate Cu^{+} and pH changes directly in the living brain, Liu et al. comparatively studied a variety of ligand structures optimized to improve the selectivity for Cu^{+}.[56] Additionally, the electrochemical probe AQ was designed and developed for the selective analysis of pH. Another type of "turn-on" electrochemical sensor as based on the enhancement of redox signals following molecular interactions recognition. Huang et al. designed and synthesized the molecule ND that specifically recognized $O_2^{\bullet-}$ and produced electroactive naphthol after chemical reactions, enabling the analysis of $O_2^{\bullet-}$ in normal and diabetic rat brain.[57] To investigate the role of H_2S_n and H_2S during oxidative stress, Dong et al. designed and synthesized two organic molecules, M_{PS-1} and M_{HS-1}, to react specifically with H_2S_n and H_2S and generated electroactive signals.[58] Luo et al. designed a series of pentamidine (DBP) derivatives that could react selectively with H_2O_2 and electroactivate the signal.[59]

Potentiometric analysis, another important electrochemical analysis method, originated from glass electrodes with a pH-sensitive response. However, the large size and leakiness of this electrode structure made it difficult to apply to brain research. Consequently, researchers designed highly selective organic ligands that were modified on the electrode surface to construct "turn-on" ion-selective microelectrodes that could monitor changes in nonelectrically active ions in real time. Recently, Zhao et al. combined small-molecule ligand recognition with PVC membrane-modified electrodes to construct microscopic-sized in vivo sensors (Figure 3D).[23] This method was successfully applied to the analysis of multiple ions during epilepsy and therapy in the living brain.

The "turn-off" type of electrochemical sensor was based on the cutoff of the electroactive fraction after the reaction of the analyte with the recognition molecule, resulting in the reduction of the original electrochemical signal. This design combined the electrochemical signal with the reactive groups to facilitate the identification of highly reactive substances in the brain. Liu et al. synthesized and designed the molecule HEMF, which underwent a specific reaction with $ONOO^{-}$

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resulting in the loss of its ferrocene groups.[60] This approach was successfully used for the analysis of $ONOO^-$ in rat brains, employing a HEMF-modified carbon fiber microelectrode. Zhang et al. developed a "turn-off" type of Fe^{2+} sensor, where the ferrocene groups on the surface of the electrode were gradually lost with increasing Fe^{2+} concentration.[61] This sensor was used for in vivo Fe^{2+} analysis in MCAO and AD models of living brains.

2.4. Reversible Recognition for Continuously Monitoring

Although recognition groups based on chemical reactions and strong ionic coordination had the advantage of being highly selective, their sensing signals were often irreversible and could not track the dynamics of a substance in real-time. To systematically investigate the reversibility of ligand recognition, Liu et al. designed and synthesized three Ca^{2+} ligands (METH, M18C6, MBAPTA) with different recognition affinities and evaluated their analytical performance. It was found that MBAPTA, which had the strongest coordination ability, was selective but poorly reversible (Figure 3E).[14] METH had a low affinity for Ca^{2+} but combined excellent reversibility and selectivity. Then, METH was modified on carbon fiber microelectrodes to develop a highly selective and reversible Ca^{2+} sensor. The sensor was applied successfully for real-time monitoring of Ca^{2+} in the brains of free-moving mice in vivo.

3. RATIOMETRIC SENSING STRATEGIES FOR QUANTITATIVE DETECTION WITH HIGH ACCURACY

The difference between in vivo and in vitro sensing was that the complex biological environment in living organisms could lead to impedance changes, ionic strength fluctuations, and nonspecific adsorption of interferents to the sensor surface. This process could lead to inaccurate single-response signals from classical electrochemical sensors, making it difficult to directly quantify the concentration of linear curves obtained in vitro for analysis. Tian's group first proposed the ratiometric electrochemical sensor (Figure 3F).[16] The sensor had a double-signal design, with the first signal exporting the electrochemical response of the analyte and the second signal being influenced only by the environment. The ratio of the two signals could provide a built-in correction. They modified bovine erythrocyte copper−zinc superoxide dismutase on the surface of electrode 1 to specifically recognize Cu^+ and 6-(ferrocenyl)hexanethiol (FcHT) on the surface of electrode 2 to provide a built-in correction as a reference signal. The sensor successfully quantified Cu^+ concentrations in a model of cerebral ischemia of mice. To reduce the error between the two working electrodes, Luo et al. designed and synthesized a new molecule, DPEA, which could recognize both Cu^{2+} and CySH.[62] This sensor combined with an internal standard signal of modified methylene blue and achieved single-electrode ratiometric electrochemical sensing. Because the advantages of the ratiometric signal sensing strategy have been demonstrated, this approach was widely used in the design of electrochemical aptamers and sensors modified with organic recognition probes. For example, Jia et al. modified an MB-labeled aptamer with an internal reference Fc on the electrode surface for highly sensitive quantitative analysis of Hg^{2+}.[63] On the other hand, an amperometric recording method known as intracellular vesicle impact electrochemical cytometry (VIEC) was developed by Ewing et al.[64] Flame-etched CF nanotips were inserted into the cytoplasm without damaging the cell membrane. The catecholamine neurotransmitters released from the vesicles on the electrode surface could then be monitored for theft. Ewing's group then further developed a single-cell amperometric recording method for quantifying catecholamine content in vesicles.[65] The vesicles were adsorbed on the electrode surface and each ruptured vesicle is subsequently observed as a current transient, allowing quantitative monitoring of catecholamine content. The next stage of enriched signals, including autoredox and electrochemiluminescence of nanomaterials, was expected to be used as ratiometric sensing strategies to further extend such self-calibrated designs to in vivo brain analysis studies.

4. STABLELY FUNCTIONALIZED AND ANTIBIOFOULING SURFACES FOR LONG-TERM RECORDING OF CHEMICAL SIGNALS

4.1. Robust Molecular Modifications on Electrode Surfaces

The functionalization of implantable electrochemical sensors is closely related to the reliable modification of molecules on the electrode surface. Ohsaka's group modified cysteine and mercaptopropionic acid on the surface of gold electrodes by Au−S bonding, which in turn self-assembled to immobilize the modified SOD.[42,43] However, Au−S was found to be easily interfered with by competing in vivo abundant thiol molecules distorting the sensor signal (Figure 4A).[66,67] Tian's group reported a highly stable H_2S_n electrochemical sensor using bidentate thiols as the linking group (Figure 4B).[68] The sensor (FP2) showed only 5.3% signal loss after 2 h immersion in glutathione (GSH) solution in artificial cerebrospinal fluid compared with a 23.4% decrease in Au−S-modified ones (FP1). The optimal adsorption modes of FP1 and FP2 on gold

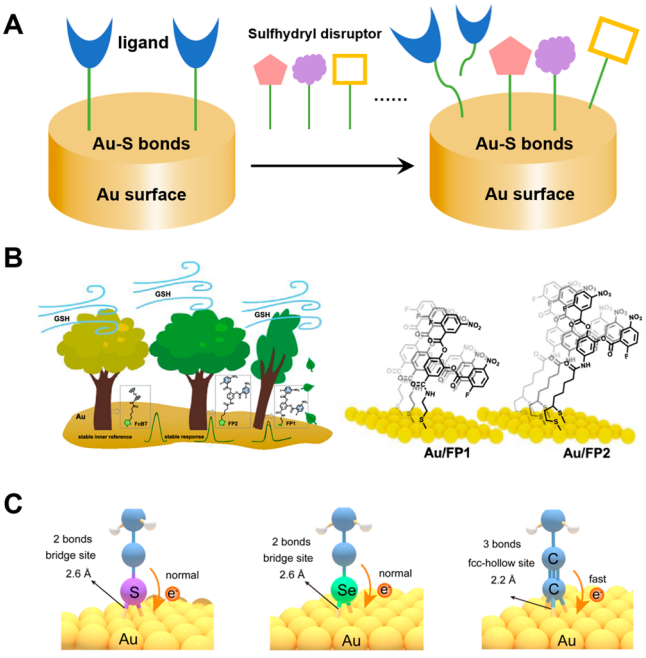

Figure 4. (A) Schematic diagram of the modified electrode based on assembled through Au−S disturbed by sulfhydryl-containing substances. (B) Schematic diagram of the sensor based on Au−S (FP1) and bidentate thiols (FP2). Reproduced with permission from ref 68. (C) Schematic diagram of the optimized gold surfaces assembled through Au−S, Au−Se, and Au−C≡C bonds. Reproduced with permission from ref 61. Copyright 2020 John Wiley and Sons.

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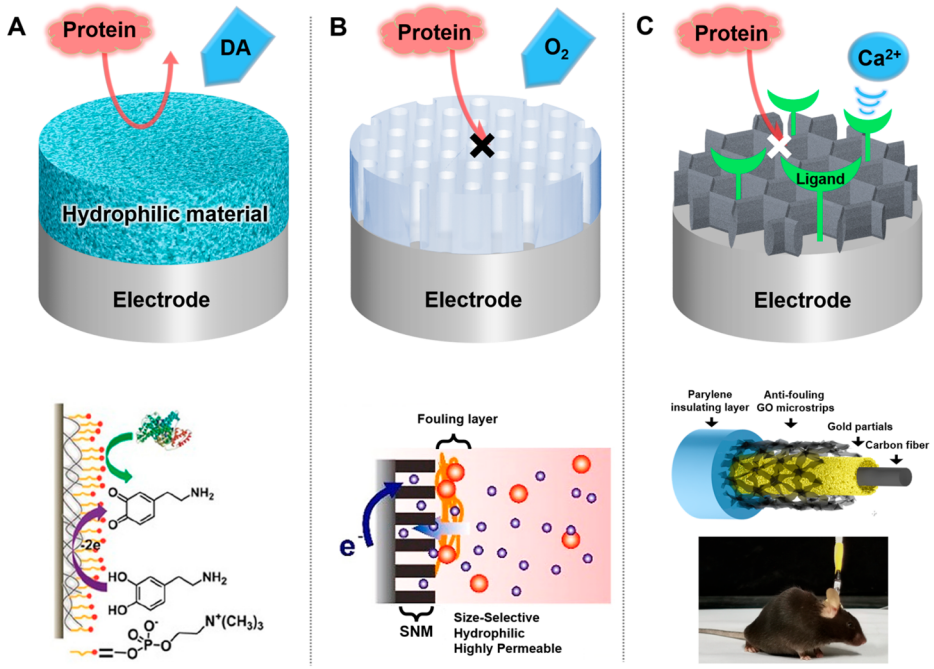

Figure 5. (A) Schematic diagram of electrode surface modified with hydrophilic material to resist biological contamination. Reproduced with permission from ref 71. Copyright 2017 John Wiley and Sons. (B) Schematic diagram of electrode modified by mesoporous material. Reproduced with permission from ref 72. (C) Schematic diagram of the resistance to biological contamination of the electrode surface interphase covered with modified graphene oxide microbands while providing recognition of molecular modification sites. Reproduced with permission from ref 14. Copyright 2021 John Wiley and Sons.

surfaces were then evaluated using integral density flooding theory (DFT). FP2 was found to be chemically bonded to the gold surface through two coordination bonds with an adsorption energy of −362.8 kJ mol^{-1}, while FP1 with coordinate sites had a weaker adsorption capacity of −275.56 kJ mol^{-1}. To evaluate the stability of multiple surface assembly methods, Zhang et al. systematically investigated formal potential ($E^{0\prime}$), peak current density (j_p), and electron transfer rate constant (k_s) of electrochemical sensors modified with Au−S, Au−Se, and Au−C≡C bonds (Figure 4C).[61] The Au−C≡C bonded surface was found to have the largest bond energy, the smallest Gibbs free energy, and the highest surface-assembled efficiency, which only showed 5.3% signal loss after 4 h of immersion in GSH solution.

4.2. Antibiofouling Surfaces for Long-Term Stability

The stability of the sensor was also related to the nonspecific adsorption of contaminants. The rapid initiation of the immune system after microelectrode implantation mainly involved the nonspecific adsorption of fibrin clots and plasma proteins produced by platelets.[69] The passivating effect of this process on the sensor could easily distort the signal. Whitesides et al. proposed empirical laws for the resistance to protein adsorption at polar functional groups and hydrophilic interfaces after studying the protein interface interaction laws.[70] This discovery has facilitated rapid advances in antibiofouling strategies. First, Mao's group proposed the electropolymerization of hydrophilic choline phosphate (PEDOT-PC) onto the electrode surface to resist protein adsorption (Figure 5A).[71] This direct coating of hydrophilic materials on the electrode surface gradually became the typical strategies. Subsequently, Su's group modified the surface of carbon fiber microelectrodes with silica nanoporous membranes to avoid protein adsorption through spatial dislocation effects (Figure 5B).[72] Although these studies significantly improved the stability of the electrodes, the full-coverage modified antipollution membranes were difficult to further modify functionalized molecules on the surface. Recently, Liu et al. electrodeposited graphene oxide microbands on the surface of carbon fiber electrodes modified with gold particles; graphene oxide microbands were well hydrophilic and negatively charged, which could be used to resist biofouling (Figure 5C).[14] This interphase distribution of hydrophilic strips not only resisted protein adsorption but also allowed the modification of functionalized ligand molecules on the intervening gold particles. The sensitivity of the sensor was maintained at 92% after 60 consecutive days of implantation for in vivo analysis.

4.3. Designing Flexible Sensors for Minimizing Biological Damage

The mechanical properties of implantable electrodes were often one of the key factors leading to biological damage and immune responses such as inflammation. Rigid electrodes could easily cause repeated "cutting" damage to brain tissue, so increasing the flexibility and reducing the size of the electrode was an efficient method to improve the biocompatibility of the sensor.[73] Advances in carbon microsensors, from metal wires with a diameter of 30 μm to carbon fiber electrodes of only around 8 μm, had led to a significant reduction in biological damage in the brain. On the other hand, implantable electrochemical sensors made of flexible polymers could also reduce bioinflammatory responses. Yang et al. developed 3D printed carbon microelectrodes and successfully analyzed DA signals in vivo.[74] Weltin et al. proposed a polymer-based flexible microsensor using a wafer-level fabrication process.[75] The sensor achieved high time-resolved analysis of glutamate in rat brain using hydrogel membrane immobilized enzymes,

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with over 75% sensitivity still present after 28 days of immersion in PBS buffer. Booth et al. extended the potential of polymer fibers.[76] They fabricated six graphite-doped electrodes inside flexible polycarbonate for simultaneous corresponding pH and lactate concentrations. The *m*-phenylenediamine exclusion layer modified on the surface of the microelectrode could effectively reduce the interference of substances such as ascorbic acid, which made the sensor successfully record the chemical signals on the surface of the mouse brain. Huang's group reported a flexible electrode with an electrostatically spun poly(3,4-ethylenedioxythiophene) (PEDOT)-based nanomesh.[77] The complete extension of cardiomyocytes on the surface of the PEDOT-based nanoweb demonstrated the good biocompatibility of the PEDOT-based nanofiber matrix, which allowed real-time monitoring of nitric oxide release and electrophysiological activity in cardiomyocytes. More recently, Huang's group developed a stretchable electrochemical device based on a gold nanotube-based double electrode.[78] The sensor is decorated with platinum nanoparticles for the analysis of NO and H_2O_2 in vascular endothelial cells.

However, flexible electrodes tend to bend during implantation and have difficulty reaching the specific deep brain regions accurately. Some research attempted to modify the surface of the flexible electrode with a rigid self-degrading material to aid implantation. Guan et al. developed a rigid PEG-adhered flexible microelectrode filament neurofluidic fringe to improve implantation performance.[79] In biocompatibility experiments, minimal neuronal cell loss was observed around the implanted neural fimbriae compared to silicone probes that caused significant neuronal cell loss after 5 weeks of implantation. With the rapid development of flexible materials, new materials such as conductive polymers, hydrogels, and two-dimensional carbon materials were expected to become substrates for implantable sensors with low biological damage in the future.

5. DEVELOPING MICROELECTRODE ARRAYS FOR SIMULTANEOUSLY MONITORING OF MULTIPLE BRAIN REGIONS

The systematic study of molecular mechanisms in the brain involved the interaction of multiple brain regions, which required sensors to monitor chemical signals from multiple brain regions simultaneously. In recent years, rapid development in materials science and microfabrication technologies supported researchers in further reducing the size of electrodes to construct electrochemical microelectrode arrays. Ledo et al. reported a ceramic-based array of multilocus platinum microelectrodes.[80] The sensor consists mainly of thin-film polycrystalline Pt and allowed monitoring of O_2 changes at multiple sites in the rat brain cortex. The above-mentioned studies mainly followed the traditional design model of the Utah array. This method enabled multilocation monitoring with tiny pins implanted directly into the brain, but it was difficult to record signals in both superficial and deep layers. Recently, a new type of Michigan array based on a silicon two-dimensional chip design had been rapidly developed. This design lithographs electrodes onto the surface to enable high throughput recording. Wei et al. developed an Au microelectrode array for simultaneous recording of cortical ascorbic acid concentration and LFP signals using microfabrication techniques based on the design of a Michigan array.[81] Zhang et al. fabricated a 16-channel silicon-based electrode chip using photolithography.[82] The chip could be implanted in the monkey brain in the midcortex and striatum for real-time analysis of dopamine concentrations and LFP signals.

Changes in multiple local electric fields when recording current signals simultaneously at different electrodes make multipoint electrochemical signal acquisition easily disturbed. Based on potentiometric sensing techniques, Zhao et al. developed microarrays that could simultaneously monitor ion concentrations at multiple sites in the brain without crosstalk (Figure 6A).[23] To investigate the relationship between Ca^{2+} in

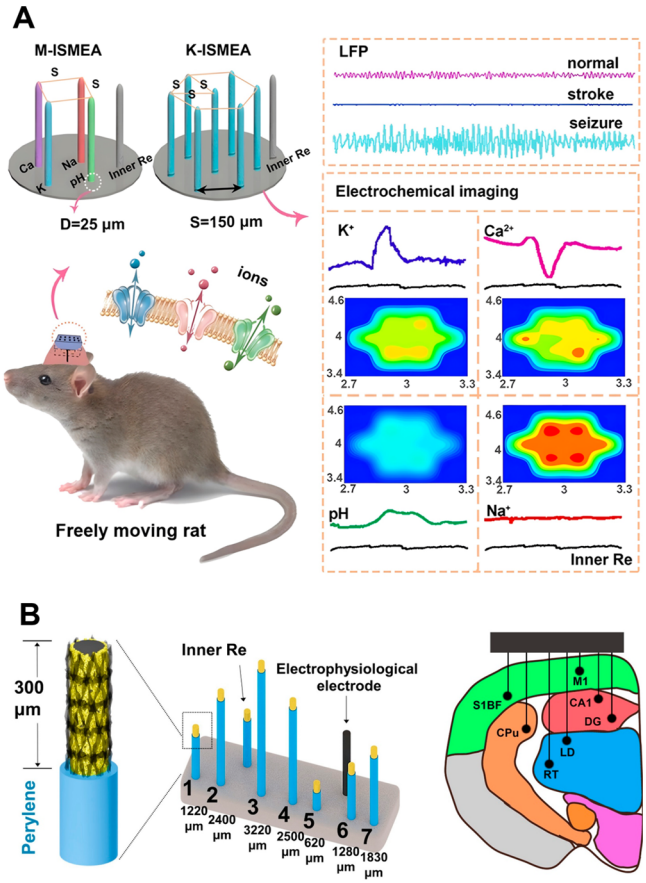

Figure 6. (A) Schematic diagram of the microarray used to simultaneously analyze K^+, Na^+, Ca^{2+}, and H^+ as well as record electrophysiological LFP signals. Reproduced with permission from ref 23. Copyright 2020 John Wiley and Sons. (B) Schematic diagram of the microarray for simultaneously recording of extracellular Ca^{2+} concentrations in seven brain regions. Reproduced with permission from ref 14. Copyright 2021 John Wiley and Sons.

multiple brain regions, Liu et al. reported a microelectrode array that could simultaneously quantify Ca^{2+} concentrations in seven brain regions and successfully discovered that Ca^{2+} changes in seven brain regions during cerebral ischemia/reperfusion were in a different order (Figure 6B).[14] Recently, nanoscale microelectrodes were developed for a wide range of applications in more in vivo electrochemical signal monitoring. For example, Huang's group developed stretchable sensors assembled from gold nanotubes, titanium dioxide nanoparticles, and carbon nanotubes. The sensor allowed real-time monitoring of intestinal 5-HT release in rats during intestinal peristalsis.[83] Ewing's group developed carbon nanopipettes of different sizes to capture intracellular vesicles and monitored the catecholamine concentration of individual vesicles.[84] However, further improvements in the spatial

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resolution of microelectrodes in the living brain still need to address key issues such as low signal-to-noise ratio and difficulty in accurate localization in complex brain environments.

6. SIMULTANEOUSLY RECORDING OF ELECTROCHEMICAL AND OTHER MULTIPLE SIGNALS IN THE LIVING BRAIN

The coupling of multiple methods in in vivo brain studies could extend the dimensionality of recorded signals and thus advance in vivo brain analysis studies. However, interferences between different methods often present compatibility issues. For example, electrophysiological techniques were the most widely used gold standard for in vivo neurobiological studies. However, this technique was so sensitive that the application of any additional voltage and current in the brain could affect the recording of neural signals. To explore the link between electrical and chemical signals, Tian's group used potentiometric analysis to construct microelectrode arrays that could be used to quantify K^+, Na^+, and Ca^{2+} concentrations and pH value in real time.[23] This applied voltage-free design first allowed the simultaneous recording of chemical and electrical signals in the living brain without interference. To simultaneously analyze intra- and extracellular molecular and electrical signals in the living brain, Tian's group developed a photophysiological probe by integrating optical and electrical signals.[85] The probe could record both CO_3^{2-} concentration in the cerebral cortex of mice and LFP sigals. Recently, they furtherly developed a Raman fiber photometry. This method quantified intracellular mitochondrial $O_2^{\bullet-}$ and Ca^{2+} as well as pH value while simultaneously recording electrophysiological and extracellular Ca^{2+} electrochemical signals.[86] Not only spectroscopic methods but also functionalized magnetic resonance imaging (fMRI) could be coupled with electrochemical microelectrode techniques. Lowry et al. simultaneously recorded blood-level-dependent (BOLD) fMRI signals and electrochemical O_2 signals from rat cerebral cortex, demonstrating the practical feasibility of electrochemical methods for obtaining real-time metabolite information during fMRI acquisition.[87] Recently, Walton et al. developed a method that allowed simultaneous FSCV and BOLD fMRI detection to record oxygen and dopamine changes and global blood oxygen levels, respectively.[88] This technique provided complementary neurochemical and blood oxygen monitoring methods. These encouraging results suggested that more in vivo analysis techniques were expected to be compatible with brain electrochemical sensors.

7. CONCLUSIONS AND OUTLOOK

The rapid development of implantable electrochemical sensors provided a reliable tool for the investigation of molecular mechanisms in the living brain. In this Perspective, we reviewed the recent progress made in the recognition performance of electrochemical sensors. The development of recognition units, such as enzymes, aptamers, and organic molecules, significantly improved the selectivity of sensors, allowing scientists to precisely resolve the mechanisms of analytes in complex brain environments. By modulating recognition groups and designing reversible sensors, real-time dynamic monitoring of chemical signals in the brain became possible. Ratiometric electrochemical sensors were designed to allow for accurate quantitative detection in complex brains. The proposed ratiometric sensing strategy made it possible to quantify the concentration of the analytes with high accuracy in the brain. Advances in biocontamination-resistant methods enabled electrochemical sensors to consistently record in the brain for extended periods. Highly biocompatible electrodes based on flexible materials reduced biological damage significantly. Finally, the coupling of multiple in vivo analytical techniques with electrochemical sensors facilitated the multidimensional recording of biosignals in the brain, improving spatial resolution and providing a more comprehensive understanding of brain function.

Despite all the advances that have been made so far with implantable electrochemical sensors, the complexity of the brain environment still poses the challenge of accurate and stable identification, for example, of structurally similar neurotransmitter molecules. In addition, it is difficult to comprehensively monitor the full range of chemical signals in the living brain using electrochemical sensors alone. Therefore, the next generation of implantable electrochemical sensors in brain research continues to be closely linked to the development of accurate identification, long-term stability, and multimethod coupling, which involves advances in molecules, materials, and devices. Supramolecular chemical recognition based on weak and reversible noncovalent interactions can be designed and modulated by molecules that hold promise for the future design of electrochemical sensors with high reversibility, fast response times, and simultaneous analysis of multiple substances. Unlike the modulation of electrical and hydrophilic modifications at the electrode interfaces, the innovation of new materials based on bionanotechnology is expected to reduce biological damage from the perspective of immune recognition, enabling highly stable in vivo analysis. The coupling of fiber optic photometric techniques and various noninvasive in vivo analytical methods such as MRI and PET with electrochemical sensors allows further insight into brain mechanisms. In addition, with the rapid development of brain−computer interfaces, implantable microelectrodes have been widely used in areas such as neuroelectric signal monitoring. However, the chemical signal changes involved in this process are difficult to record in real time. Functionalized electrochemical microelectrode technology is expected to access clinical brain−computer interface tests and expand the dimensionality of in vivo analysis signals. Also, implantable electrochemical sensors are expected to be used directly in human in vivo analytical procedures, such as chemical signal monitoring during surgery, in the future after further reducing biological losses and improving detection accuracy. As a new mode of brain research in vivo, implantable electrochemical sensors combined with various detection methods will become a reliable tool for studying brain mechanisms in the future.

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Notes

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■ REFERENCES

(1) Langen, K.; Galldiks, N.; Hattingen, E.; Shah, N. Advances in neuro-oncology imaging. *Nat. Rev. Neurol.* **2017**, *13*, 279−289.
(2) Zhang, Q.; Tian, Y. Rapid and specific detection of norepinephrine via a "hunting-shooting" strategy. *Sci. China Chem.* **2023**, *66*, 923−925.
(3) Liu, Z.; Zhu, Y.; Zhang, L.; Jiang, W.; Liu, Y.; Tang, Q.; Cai, X.; Li, J.; Wang, L.; Tao, C.; Yin, X.; Li, X.; Hou, S.; Jiang, D.; Liu, K.; Zhou, X.; Zhang, H.; Liu, M.; Fan, C.; Tian, Y. Structural and functional imaging of brains. *Sci. China Chem.* **2023**, *66*, 324−366.
(4) Zhang, L.; Tian, Y. Designing Recognition Molecules and Tailoring Functional Surfaces for In Vivo Monitoring of Small Molecules in the Brain. *Acc. Chem. Res.* **2018**, *51* (3), 688−696.
(5) Yin, H.; Jiang, W.; Liu, Y.; Zhang, D.; Wu, F.; Zhang, Y.; Li, C.; Chen, G.; Wang, Q. Advanced near-infrared light approaches for neuroimaging and neuromodulation. *BMEMat* **2023**.
(6) Li, C.; Chen, G.; Zhang, Y.; Wu, F.; Wang, Q. Advanced Fluorescence Imaging Technology in the near-Infrared-Ii Window for Biomedical Applications. *J. Am. Chem. Soc.* **2020**, *142*, 14789−14804.
(7) Liu, Z.; Tian, Y. Recent advances in development of devices and probes for sensing and imaging in the brain. *Sci. China Chem.* **2021**, *64*, 915−931.
(8) Liu, Y.; Liu, Z.; Tian, Y. Real-Time Tracking of Electrical Signals and an Accurate Quantification of Chemical Signals with Long-Term Stability in the Live Brain. *Acc. Chem. Res.* **2022**, *55* (19), 2821−2832.
(9) Gong, Z.; Liu, Z.; Zhang, Z.; Mei, Y.; Tian, Y. A Highly Stable Two-Photon Ratiometric Fluorescence Probe for Real-Time Biosensing and Imaging of Nitric Oxide in Brain Tissues and Larval Zebrafish. *CCS Chem.* **2022**, *4*, 1−23.
(10) Robinson, D.; Hermans, A.; Seipel, A.; Wightman, R. Monitoring rapid chemical communication in the brain. *Chem. Rev.* **2008**, *108*, 2554−2584.
(11) Wang, S.; Liu, Y.; Zhu, A.; Tian, Y. In Vivo Electrochemical Biosensors: Recent Advances in Molecular Design, Electrode Materials, and Electrochemical Devices. *Anal. Chem.* **2023**, *95* (1), 388−406.
(12) Zhou, Q.; Zhang, L.; Tian, Y. Advanced electrochemical strategy for in vivo detection of electrochemically inactive molecules. *J. Electrochem.* **2019**, *25*, 160−171.
(13) Da, Y.; Luo, S.; Tian, Y. Real-Time Monitoring of Neurotransmitters in the Brain of Living Animals. *ACS Appl. Mater. Interfaces* **2023**, *15* (1), 138−157.
(14) Liu, Y.; Liu, Z.; Zhao, F.; Tian, Y. Long-term tracking and dynamically quantifying of reversible changes of extracellular Ca^{2+} in multiple brain regions of freely moving animals. *Angew. Chem., Int. Ed.* **2021**, *60*, 14429−14437.
(15) Tian, Y.; Mao, L.; Ohsaka, T. Electrochemical biosensors for superoxide anion. *Current Anal. Chem.* **2006**, *2*, 51−58.
(16) Zhang, L.; Han, Y.; Zhao, F.; Shi, G.; Tian, Y. A selective and accurate ratiometric electrochemical biosensor for monitoring of Cu^{2+} ions in a rat brain. *Anal. Chem.* **2015**, *87*, 2931−2936.
(17) Ding, S.; Cao, S.; Liu, Y.; Lian, Y.; Zhu, A.; Shi, G. Rational design of a stimuli-responsive polymer electrode interface coupled with in vivo microdialysis for measurement of sialic acid in live mouse brain in Alzheimer's disease. *ACS Sens.* **2017**, *2*, 394−400.
(18) Chai, X.; Zhou, X.; Zhu, A.; Zhang, L.; Qin, Y.; Shi, G.; Tian, Y. A two-channel ratiometric electrochemical biosensor for in vivo monitoring of copper ions in a rat brain using gold truncated octahedral microcages. *Angew. Chem., Int. Ed.* **2013**, *52*, 8129−8133.
(19) Harreither, W.; Trouillon, R.; Poulin, P.; Neri, W.; Ewing, A. G.; Safina, G. Carbon Nanotube Fiber Microelectrodes Show a Higher Resistance to Dopamine Fouling. *Anal. Chem.* **2013**, *85*, 7447−7453.
(20) Lowe, S.; O'Brien-Simpson, N.; Connal, L. Antibiofouling polymer interfaces: poly(ethylene glycol) and other promising candidates. *Polym. Chem.* **2015**, *6*, 198−212.
(21) Emilsson, G.; Schoch, R.; Feuz, L.; Höök, F.; Lim, R.; Dahlin, A. Strongly stretched protein resistant poly(ethylene glycol) brushes prepared by grafting-to. *ACS Appl. Mater. Interfaces* **2015**, *7*, 7505−7515.
(22) Liu, Y.; Zhang, L.; Mo, J.; Zhou, Y.; Tian, Y. Ion Selective Electrode Mediated by Transfer Layer of Graphene Oxide for Detection of Ca^{2+} with Highly Sensitivity and Selectivity. *J. Electrochem. Soc.* **2022**, *169*, 016514.
(23) Zhao, F.; Liu, Y.; Dong, H.; Feng, S.; Shi, G.; Lin, L.; Tian, Y. An electrochemophysiological microarray for real-time monitoring and quantification of multiple ions in the brain of a freely moving rat. *Angew. Chem., Int. Ed.* **2020**, *59*, 10426−10430.
(24) Kissinger, P.; Hart, J.; Adams, R. Voltammetry in brain tissue — a new neurophysiological measurement. *Brain Res.* **1973**, *55* (1), 209−213.
(25) Wightman, R.; May, L.; Michael, A. Detection of dopamine dynamics in the brain. *Anal. Chem.* **1988**, *60* (13), 769A−779A.
(26) Phillips, P. E. M.; Stuber, G. D.; Heien, M.; Wightman, R. M.; Carelli, R. M. Subsecond Dopamine Release Promotes Cocaine Seeking. *Nature* **2003**, *422*, 614−618.
(27) Clark, L. C.; Lyons, C. Electrode systems for continuous monitoring in cardiovascular surgery. *Ann. N. Y. Acad. Sci.* **1962**, *102*, 29−45.
(28) Baker, K.; Bolger, F.; Lowry, J. A microelectrochemical biosensor for real-time in vivo monitoring of brain extracellular choline. *Analyst* **2015**, *140*, 3738−3745.
(29) Cass, A.; Davis, G.; Francis, G.; Hill, H.; Aston, W.; Higgins, I.; Plotkin, E.; Scott, L.; Turner, A. Ferrocene-mediated enzyme electrode for amperometric determination of glucose. *Anal. Chem.* **1984**, *56*, 667−671.
(30) Hu, J.; Turner, A. An enzyme electrode for glucose consisting of gucose oxidase immobilised at a benzoquinone-modified carbon electrode. *Anal. Lett.* **1991**, *24*, 15−24.
(31) Forrow, N.; Walters, S. Transition metal half-sandwich complexes as redox mediators to glucose oxidase. *Biosens. Bioelectron.* **2004**, *19*, 763−770.
(32) Bartlett, P.; Booth, S.; Caruana, D.; Kilburn, J.; Santamaria, C. Modification of glucose oxidase by the covalent attachment of a tetrathiafulvalene derivative. *Anal. Chem.* **1997**, *69*, 734−742.

(33) Wang, X.; Li, Q.; Xu, J.; Wu, S.; Xiao, T.; Hao, J.; Yu, P.; Mao, L. Rational design of bioelectrochemically multifunctional film with oxidase, ferrocene, and graphene oxide for development of in vivo electrochemical biosensors. *Anal. Chem.* **2016**, *88*, 5885−5891.
(34) Taniguchi, I.; Toyosawa, K.; Yamaguchi, H.; Yasukouchi, K. Voltammetric response of horse heart cytochrome c at a gold electrode in the presence of sulfur bridged bipyridines. *J. Electroanal. Chem.* **1982**, *140*, 187−193.
(35) Liu, H.; Tian, Y.; Deng, Z. Morphology-dependent electrochemistry and electrocatalytical activity of cytochrome c. *Langmuir* **2007**, *23*, 9487−9494.
(36) Deng, Z.; Gong, Y.; Luo, Y.; Tian, Y. WO_3 nanostructures facilitate electron transfer of enzyme: Application to detection of H_2O_2 with high selectivity. *Biosens. Bioelectron.* **2009**, *24*, 2465−2469.
(37) Zhu, A.; Luo, Y.; Tian, Y. Plasmon-Induced Enhancement in Analytical Performance Based on Gold Nanoparticles Deposited on TiO_2 Film. *Anal. Chem.* **2009**, *81* (17), 7243−7247.
(38) Zhu, A.; Tian, Y.; Liu, Y.; Luo, Y. Nanoporous gold film encapsulating cytochrome c for the fabrication of a H_2O2 biosensor. *Biomaterials* **2009**, *30*, 3183−3188.
(39) Luo, Y.; Liu, H.; Rui, Q.; Tian, Y. Detection of Extracellular H_2O2 Released from Human Liver Cancer Cells Based on TiO_2 Nanoneedles with Enhanced Electron Transfer of Cytochrome c. *Anal. Chem.* **2009**, *81* (8), 3035−3041.
(40) Li, X.; Liu, Y.; Zhu, A.; Luo, Y.; Deng, Z.; Tian, Y. Real-Time Electrochemical Monitoring of Cellular H_2O2 Integrated with In Situ Selective Cultivation of Living Cells Based on Dual Functional Protein Microarrays at Au−TiO_2 Surfaces. *Anal. Chem.* **2010**, *82* (15), 6512−6518.
(41) Zhou, J.; Liao, C.; Zhang, L.; Wang, Q.; Tian, Y. Molecular hydrogel-stabilized enzyme with facilitated electron transfer for determination of H_2O_2 released from live cells. *Anal. Chem.* **2014**, *86*, 4395−4401.
(42) Tian, Y.; Mao, L.; Okajima, T.; Ohsaka, T. Superoxide dismutase-based third-generation biosensor for superoxide anion. *Anal. Chem.* **2002**, *74*, 2428−2434.
(43) Tian, Y.; Mao, L.; Okajima, T.; Ohsaka, T. Electrochemistry and electrocatalytic activities of superoxide dismutases at gold electrodes modified with a self-assembled monolayer. *Anal. Chem.* **2004**, *76*, 4162−4168.
(44) Xia, P.; Liu, H.; Tian, Y. Cathodic detection of H_2O_2 based on nanopyramidal gold surface with enhanced electron transfer of myoglobin. *Biosens. Bioelectron.* **2009**, *24*, 2470−2474.
(45) Rui, Q.; Komori, K.; Tian, Y.; Liu, H.; Luo, Y.; Sakai, Y. Electrochemical biosensor for the detection of H_2O_2 from living cancer cells based on ZnO nanosheets. *Anal. Chim. Acta* **2010**, *670*, 57−62.
(46) Deng, Z.; Rui, Q.; Yin, X.; Liu, H.; Tian, Y. In vivo detection of superoxide anion in bean sprout based on ZnO nanodisks with facilitated activity for direct electron transfer of superoxide dismutase. *Anal. Chem.* **2008**, *80*, 5839−5846.
(47) Wang, Z.; Liu, D.; Gu, H.; Zhu, A.; Tian, Y.; Shi, G. NTA-modified carbon electrode as a general relaying substrate to facilitate electron transfer of SOD: application to in vivo monitoring of $O_2^{\cdot -}$ in a rat brain. *Biosens. Bioelectron.* **2013**, *43*, 101−107.
(48) Luo, Y.; Tian, Y.; Rui, Q. Electrochemical assay of superoxide based on biomimetic enzyme at highly conductive TiO_2 nanoneedles: from principle to applications in living cells. *Chem. Commun.* **2009**, *21*, 3014−3017.
(49) Marrazza, G. Aptamer Sensors. *Biosensors* **2017**, *7*, 5.
(50) Liu, M.; Zhang, X.; Huang, L.; Li, J.; Fan, C.; Tian, Y. DNA Nanostructure-Guided Plasmon Coupling Architectures. *CCS Chemistry* **2023**, *5*, 568−588.
(51) Zuo, X.; Song, S.; Zhang, J.; Pan, D.; Wang, L.; Fan, C. A Target-Responsive Electrochemical Aptamer Switch (TREAS) for Reagentless Detection of Nanomolar ATP. *J. Am. Chem. Soc.* **2007**, *129* (5), 1042−1043.
(52) Wen, Y.; Pei, H.; Wan, Y.; Su, Y.; Huang, Q.; Song, S.; Fan, C. DNA Nanostructure-Decorated Surfaces for Enhanced Aptamer-Target Binding and Electrochemical Cocaine Sensors. *Anal. Chem.* **2011**, *83* (19), 7418−7423.
(53) Wang, P.; Wan, Y.; Deng, S.; Yang, S.; Su, Y.; Fan, C.; Aldalbahi, A.; Zuo, X. Aptamer-initiated on-particle template-independent enzymatic polymerization (aptamer-OTEP) for electrochemical analysis of tumor biomarkers. *Biosens. Bioelectron.* **2016**, *86* (15), 536−541.
(54) Qiu, Z.; Shu, J.; Liu, J.; Tang, D. Dual-Channel Photoelectrochemical Ratiometric Aptasensor with up-Converting Nanocrystals Using Spatial-Resolved Technique on Homemade 3D Printed Device. *Anal. Chem.* **2019**, *91* (2), 1260−1268.
(55) Zhang, H.; Wang, Z.; Wang, F.; Zhang, Y.; Wang, H.; Liu, Y. In Situ Formation of Gold Nanoparticles Decorated Ti3C2MXenes Nanoprobe for Highly Sensitive Electrogenerated Chemiluminescence Detection of Exosomes and Their Surface Proteins. *Anal. Chem.* **2020**, *92* (7), 5546−5553.
(56) Liu, W.; Dong, H.; Zhang, L.; Tian, Y. Development of an efficient biosensor for the in vivo monitoring of Cu^+ and pH in the brain: Rational design and synthesis of recognition molecules. *Angew. Chem. Int. Ed.* **2017**, *56*, 16328−16332.
(57) Huang, S.; Zhang, L.; Dai, L.; Wang, Y.; Tian, Y. Nonenzymatic electrochemical sensor with ratiometric signal output for selective determination of superoxide anion in rat brain. *Anal. Chem.* **2021**, *93*, 5570−5576.
(58) Dong, H.; Zhou, Q.; Zhang, L.; Tian, Y. Rational design of specific recognition molecules for simultaneously monitoring of endogenous polysulfide and hydrogen sulfide in the mouse brain. *Angew. Chem., Int. Ed.* **2019**, *58*, 13948−13953.
(59) Luo, Y.; Lin, R.; Zuo, Y.; Zhang, Z.; Zhuo, Y.; Lu, M.; Chen, S.; Gu, H. Efficient electrochemical microsensor for in vivo monitoring of H_2O_2 in PD mouse brain: Rational design and synthesis of recognition molecules. *Anal. Chem.* **2022**, *94*, 9130−9139.
(60) Liu, F.; Dong, H.; Tian, Y. Real-time monitoring of peroxynitrite ($ONOO^-$) in the rat brain by developing a ratiometric electrochemical biosensor. *Analyst* **2019**, *144*, 2150−2157.
(61) Zhang, C.; Liu, Z.; Zhang, L.; Zhu, A.; Liao, F.; Wan, J.; Zhou, J.; Tian, Y. A Robust Au−C≡C Functionalized Surface: Toward Real-Time Mapping and Accurate Quantification of Fe^{2+} in the Live Brains of AD Mouse Models. *Angew. Chem., Int. Ed.* **2020**, *59* (46), 20499−20507; *Angew. Chem.* **2020**, *132* (46), 20680−20688.
(62) Luo, Y.; Zhang, L.; Liu, W.; Yu, Y.; Tian, Y. A single biosensor for evaluating the levels of copper ion and L-cysteine in a live rat brain with Alzheimer's disease. *Angew. Chem., Int. Ed.* **2015**, *54*, 14053−14056; *Angew. Chem.* **2015**, *127*, 14259−14262.
(63) Jia, J.; Liu, Z.; Xiao, X.; Liu, B.; Chou, K. iPPBS-Opt: A Sequence-Based Ensemble Classifier for Identifying Protein-Protein Binding Sites by Optimizing Imbalanced Training Datasets. *Molecules* **2016**, *21* (1), 95.
(64) Li, X.; Dunevall, J.; Ren, L.; Ewing, A. G. Mechanistic Aspects of Vesicle Opening during Analysis with Vesicle Impact Electrochemical Cytometry. *Anal. Chem.* **2017**, *89*, 9416−9423.
(65) Hu, K.; Jia, R.; Hatamie, A.; Le Vo, K. L.; Mirkin, M. V.; Ewing, A. G. Correlating Molecule Count Release Kinetics with Vesicular Size Using Open Carbon Nanopipettes. *J. Am. Chem. Soc.* **2020**, *142* (40), 16910−16914.
(66) Liu, H.; Radford, M. R.; Yang, C.; Chen, W.; Xian, M. Inorganic Hydrogen Polysulfides: Chemistry, Chemical Biology, and Detection. *Br. J. Pharmacol.* **2019**, *176*, 616−627.
(67) Hu, B.; Kong, F.; Gao, X.; Jiang, L.; Li, X.; Gao, W.; Xu, K.; Tang, B. Avoiding Thiol Compound Interference: A Nanoplatform Based on High-Fidelity Au-Se Bonds for Biological Applications. *Angew. Chem., Int. Ed.* **2018**, *57*, 5306−5309; *Angew. Chem.* **2018**, *130*, 5404−5407.
(68) Qian, Y.; Zhang, L.; Tian, Y. Highly stable electrochemical probe with bidentate thiols for ratiometric monitoring of endogenous polysulfide in living mouse brains. *Anal. Chem.* **2022**, *94*, 1447−1455.
(69) Liu, Y.; Liu, J.; Chen, S.; Lei, T.; Kim, Y.; Niu, S.; Wang, H.; Wang, X.; Foudeh, A.; Tok, J.; Bao, Z. Soft and elastic hydrogel-based

https://doi.org/10.1021/jacsau.3c00200
JACS Au 2023, 3, 1572−1582

microelectronics for localized low-voltage neuromodulation. *Nat. Biomed. Eng.* **2019**, *3*, 58−68.
(70) Wei, Q.; Becherer, T.; Angioletti-Uberti, S.; Dzubiella, J.; Wischke, C.; Neffe, A. T.; Lendlein, A.; Ballauff, M.; Haag, R. Protein Interactions with Polymer Coatings and Biomaterials. *Angew. Chem., Int. Ed.* **2014**, *53*, 8004−8031.
(71) Liu, X.; Xiao, T.; Wu, F.; Shen, M.; Zhang, M.; Yu, H.; Mao, L. Ultrathin Cell-Membrane-Mimic Phosphorylcholine Polymer Film Coating Enables Large Improvements for InVivo Electrochemical Detection. *Angew. Chem., Int. Ed.* **2017**, *56*, 11802−11806.
(72) Zhou, L.; Hou, H.; Wei, H.; Yao, L.; Sun, L.; Yu, P.; Su, B.; Mao, L. In Vivo Monitoring of Oxygen in Rat Brain by Carbon Fiber Microelectrode Modified with Antifouling Nanoporous Membrane. *Anal. Chem.* **2019**, *91*, 3645−3651.
(73) Chatard, C.; Sabac, A.; Moreno-Velasquez, L.; Meiller, A.; Marinesco, S. Minimally Invasive Microelectrode Biosensors Based on Platinized Carbon Fibers for in Vivo Brain Monitoring. *ACS Cent. Sci.* **2018**, *4*, 1751−1760.
(74) Yang, C.; Cao, Q.; Puthongkham, P.; Lee, S. T.; Ganesana, M.; Lavrik, N. V.; Venton, B. J. 3D-Printed Carbon Electrodes for Neurotransmitter Detection. *Angew. Chem., Int. Ed.* **2018**, *57*, 14255−14259.
(75) Weltin, A.; Kieninger, J.; Enderle, B.; Gellner, A.; Fritsch, B.; Urban, G. Polymer-based, flexible glutamate and lactate microsensors for in vivo applications. *Biosens. Bioelectron.* **2014**, *61*, 192−199.
(76) Booth, M.; Gowers, S.; Hersey, M.; Samper, I.; Park, S.; Anikeeva, P.; Hashemi, P.; Stevens, M.; Boutelle, M. Fiber-Based Electrochemical Biosensors for Monitoring pH and Transient Neurometabolic Lactate. *Anal. Chem.* **2021**, *93* (17), 6646−6655.
(77) Yan, Li.; Wen, M.; Qin, Y.; Bi, C.; Zhao, Y.; Fan, W.; Yan, J.; Huang, W.; Liu, Y. Soft Electrodes for Electrochemical and Electrophysiological Monitoring of Beating Cardiomyocytes. *Angew. Chem., Int. Ed.* **2022**, *61* (26), e202203757.
(78) Fan, W.; Zhao, Y.; Wu, W.; Qin, Y.; Yan, J.; Liu, Y.; Huang, W. Redox Homeostasis Alteration in Endothelial Mechanotransduction Monitored by Dual Stretchable Electrochemical Sensors. *Anal. Chem.* **2022**, *94* (20), 7425−7432.
(79) Guan, S.; Wang, J.; Gu, X.; Zhao, Y.; Hou, R.; Fan, H.; Zou, L.; Gao, L.; Du, M.; Li, C.; Fang, Y. Elastocapillary Self-Assembled Neurotassels for Stable Neural Activity Recordings. *Sci. Adv.* **2019**, *5*, eaav2842.
(80) Ledo, A.; Lourenco, C. F.; Laranjinha, J.; Gerhardt, G. A.; Barbosa, R. M. Combined In Vivo Amperometric Oximetry and Electrophysiology in a Single Sensor: A Tool for Epilepsy Research. *Anal. Chem.* **2017**, *89*, 12383−12390.
(81) Wei, H.; Li, L.; Jin, J.; Wu, F.; Yu, P.; Ma, F.; Mao, L. Galvanic Redox Potentiometry Based Microelectrode Array For Synchronous Ascorbate And Single-Unit Recordings In Rat Brain. *Anal. Chem.* **2020**, *92*, 10177−10182.
(82) Zhang, S.; Song, Y.; Wang, M.; Zhang, Z.; Fan, X.; Song, X.; Zhuang, P.; Yue, F.; Chan, P.; Cai, X. A silicon based implantable microelectrode array for electrophysiological and dopamine recording from cortex to striatum in the non-human primate brain. *Biosens. Bioelectron.* **2016**, *85*, 53−61.
(83) Qi, Y.; Zhang, F.; Tian, S.; Yang, X.; Liu, Y.; Huang, W. Construction of micro-nano electrochemical sensing interfaces for real-time monitoring of single cells. *Sci. China Chem.* **2021**, *51* (3), 359−373.
(84) Hu, K.; Le Vo, K. L.; Hatamie, A.; Ewing, A. G. Quantifying Intracellular Single Vesicular Catecholamine Concentration with Open Carbon Nanopipettes to Unveil the Effect of L-DOPA on Vesicular Structure. *Angew. Chem., Int. Ed.* **2022**, *61*, e202113406.
(85) Wang, W.; Zhao, F.; Li, M.; Zhang, C.; Shao, Y.; Tian, Y. A SERS Optophysiological Probe for the Real-Time Mapping and Simultaneous Determination of the Carbonate Concentration and pH Value in a Live Mouse Brain. *Angew. Chem., Int. Ed.* **2019**, *58* (16), 5256−5260; *Angew. Chem.* **2019**, *131* (16), 5310−5314.
(86) Liu, Z.; Zhang, Z.; Liu, Y.; Mei, Y.; Feng, E.; Liu, Y.; Zheng, T.; Chen, J.; Zhang, S.; Tian, Y. Raman Fiber Photometry for Understanding Mitochondrial Superoxide Burst and Extracellular Calcium Ion Influx upon Acute Hypoxia in the Brain of Freely Moving Animals. *Angew. Chem., Int. Ed.* **2022**, *61* (11), e202111630; *Angew. Chem.* **2022**, *134* (11), e202111630.
(87) Lowry, J. P.; Griffin, K.; McHugh, S. B.; Lowe, A. S.; Tricklebank, M.; Sibson, N. R. Real-time electrochemical monitoring of brain tissue oxygen: a surrogate for functional magnetic resonance imaging in rodents. *NeuroImage* **2010**, *52* (2010), 549−555.
(88) Walton, L. R.; Verber, M.; Lee, S.-H.; Chao, T.-H. H.; Wightman, R. M.; Shih, Y.-Y. I. Simultaneous fMRI and fast-scan cyclic voltammetry bridges evoked oxygen and neurotransmitter dynamics across spatiotemporal scales. *NeuroImage* **2021**, *244*, 118634.

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